The Effect of Long-Term Intake of cis Unsaturated Fats on the Risk for Gallstone Disease in Men

A Prospective Cohort Study

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Background: **Monounsaturated and polyunsaturated fats act as inhibitors of cholesterol cholelithiasis in animal experiments.**

Objective: **To examine the association between long-term intake of cis unsaturated fats and the incidence of gallstone disease in humans.**

Design: **Prospective population-based cohort study.**

Setting: **The Health Professional Follow-up Study.**

Participants: **45 756 men, age 40 to 75 years in 1986, who were free of gallstone disease.**

Measurements: **Consumption of cis unsaturated fats was assessed starting in 1986 as part of the 131-item semi-quantitative food-frequency questionnaires. Questionnaires were mailed to participants every 2 years. The main outcome measure was self-reported newly diagnosed symptomatic gallstone disease.**

Results: **During 14 years of follow-up, 2323 new cases of gallstone disease were documented. After adjustment for age and other potential risk factors, the relative risk for gallstone disease among men in the highest quintile of dietary intake of cis unsaturated fats compared with men in the lowest quintile was 0.82 (95% CI, 0.69 to 0.96; *P* for trend = 0.006). The relative risk among men in the highest quintile of polyunsaturated fat consumption compared with men in the lowest quintile was 0.84 (CI, 0.73 to 0.96; *P* for trend = 0.01), and the relative risk among men in the highest quintile of monounsaturated fat consumption compared with men in the lowest quintile was 0.83 (CI, 0.70 to 1.00; *P* for trend = 0.01).**

Limitations: **Outcomes were restricted to men with cholecystectomy or diagnostically confirmed but unremoved symptomatic gallstones.**

Conclusions: **A high intake of polyunsaturated and monounsaturated fats in the context of an energy-balanced diet is associated with a reduced risk for gallstone disease in men.**

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For definitions of terms used in the text, see Glossary.

Gallbladder disease is a major source of morbidity in the United States. It affects approximately 10% to 25% of U.S. adults (1). Each year, more than 800 000 hospitalizations in the United States are attributed to gallstone disease (2). In most western countries, including the United States, cholesterol gallstones account for approximately 80% to 90% of the stones removed in patients between the ages of 30 and 60 years (3). Thus, preventive measures to reduce the incidence of gallstones may decrease the burden of gallbladder disease.

Cholesterol gallstones have many causes; one of the most important causes is hypersecretion of cholesterol into the biliary tree (4). Hyperinsulinemia has been associated with increased bile cholesterol saturation and may enhance cholesterol gallstone formation (5), and gallstone disease is thought to be a manifestation of the insulin resistance syndrome (6). Studies report (7, 8) that diets high in polyunsaturated and monounsaturated fatty acids (see Glossary) relative to low-fat, high-carbohydrate diets have beneficial effects on insulin sensitivity. In addition, in animal experiments, monounsaturated and polyunsaturated fats can inhibit cholesterol cholelithiasis and, therefore, may protect against cholesterol gallstone disease in humans (9–11). However, earlier epidemiologic studies on the relationship between consumption of polyunsaturated and monounsaturated fatty acids and risk for gallstone disease are sparse and provide no convincing evidence (12–16). Published studies have been limited by inadequate dietary assessment, short follow-up periods, incomplete control of confounding, or small number of end points. In cross-sectional or case–control studies, changes in diet caused by symptoms of gallstones could influence assessment of dietary intake.

We therefore examined dietary intakes of monounsaturated and polyunsaturated fatty acids in relation to the occurrence of gallstone disease in a large cohort of U.S. male health professionals.

METHODS

Study Sample

The Health Professionals Follow-up Study began in 1986 when 51 529 U.S. male dentists (58%), veterinarians (20%), optometrists (7%), osteopathic physicians (4%), and podiatrists (3%) who were 40 to 75 years of age returned a questionnaire by mail regarding diet, medications, and medical history. Follow-up questionnaires were sent every 2 years to update information on exposures and to ascertain the occurrence of newly diagnosed illnesses, including gallstone disease. Diet was assessed in 1986, 1990, 1994, and 1998. At baseline, we excluded men who reported a cholecystectomy or a diagnosis of gallstone disease before 1986, men with a diagnosis of cancer before 1986, men with a daily energy intake outside the range of 800 to 4200 kcal/d, and men with 70 or more blank food items on the questionnaire. After exclusions, the study sample consisted of 45 756 eligible men who were followed from

1986 to 2000. The average follow-up rate for biennial questionnaires was greater than 94% in each 2-year follow-up cycle.

Diet Assessment

Dietary information was derived from a 131-item semi-quantitative food-frequency questionnaire (17). We asked participants to indicate the frequency, on average, of consuming a typical serving size of selected foods during the previous year. Respondents could choose from 9 options, ranging from never or less than once per month to 6 or more times per day. Nutrient scores were computed by multiplying the frequency of consumption of each unit of food from the semi-quantitative food-frequency questionnaire by the nutrient content of the specified portion according to food composition tables (18). Previous reports have described the semi-quantitative food-frequency questionnaire, including fat intake and the procedures used for calculating nutrient and caloric intake, as well as data on reproducibility and validity in this cohort (19, 20). The validity of the semi-quantitative food-frequency questionnaire was assessed in a random sample of 127 participants living in the Boston, Massachusetts, area. We compared nutrient intakes from the semi-quantitative food-frequency questionnaire with 2 detailed 1-week diet records spaced approximately 6 months apart; all foods consumed each day were recorded on these records (17). The Pearson correlation coefficient, adjusted for energy and corrected for within-person variation, between the diet records and the dietary questionnaire was 0.75 for saturated fat (see Glossary), 0.76 for cholesterol, 0.68 for monounsaturated fat, and 0.37 for polyunsaturated fat. All nutrients were adjusted for total energy intake by using regression analysis.

Glossary

Monounsaturated fatty acids: At 1 point along the carbon backbone, 2 carbons are connected by a double bond. This double bond changes the shape of the molecule from a straight chain to a bent stick, and it makes the fat a liquid at room temperature. Basically, monounsaturated fats are oils. Olive oil, peanut oil, canola oil, avocados, and most nuts are all high in monounsaturated fats.

Polyunsaturated fatty acids: Two or more double bonds make a polyunsaturated fat. They are found mainly in vegetable oils, such as safflower, corn, canola oils, and fatty fish, such as salmon and tuna. Polyunsaturated fats can be subdivided into the n-3 or n-6 groups. Each type plays different roles in the body. They are liquid at room temperature. Our bodies do not make polyunsaturated fats, so they must be obtained from foods we eat.

Saturated fatty acids: A fatty acid saturated with hydrogen atoms. About 2 dozen different saturated fats exist in nature. They are found chiefly in animal sources, such as meat and poultry and dairy products. Some vegetable oils, such as coconut and palm oil, are saturated. Saturated fats are usually solid at room temperature.

Trans fatty acids: Formed through partial hydrogenation of unsaturated oils. During the process, hydrogen latches on to some of the double-bonded carbons, changing them into single bonds. It makes polyunsaturated oils as solids or semisolids, which are easier to ship and to store. Trans fats are used in many snacks, such as cookies and fried foods, and in margarine.

Context

Some people believe that high dietary intakes of cis unsaturated fat decrease cholesterol gallstone disease.

Contribution

This prospective, population-based study involving 45 756 middle-aged and older men found that during 14 years of follow-up, high compared with low intakes of both monounsaturated and polyunsaturated fatty acids were associated with fewer self-reported cases of symptomatic gallstone disease.

Cautions

The study compared high and low intakes of fatty acids; it did not determine optimal amounts of unsaturated fat intake.

Implications

Long-term intake of high amounts of total dietary cis unsaturated fat seems to be associated with decreased risk for gallstone disease in men.

–The Editors

This approach is based on the concept that the composition of the diet, independent of total energy intake, is most relevant to dietary recommendations (19). In addition, control for calorie intake can limit misclassification in nutrient intake caused by differences in body size and physical activity level.

Ascertainment of End Points

The primary end point was incident symptomatic gallstone disease. In 1986 and on each follow-up questionnaire every 2 years, participants were asked whether they had undergone cholecystectomy or had received (from a physician) a diagnosis of gallstones. Participants were also asked whether the gallstone diagnosis had been confirmed by radiographic procedures or surgery and whether their gallstones were symptomatic. To verify the self-reports of surgical cholecystectomy and diagnosed but unremoved gallstones, a random sample of 441 medical records of participants who reported a cholecystectomy or gallstones were reviewed; of these, the diagnosis was confirmed in all but 5 (99%). In addition, medical chart review confirmed all self-reported symptoms and all but 1 of the self-reported diagnostic procedures.

Statistical Analysis

For each participant, follow-up time accrued from the month of return of the 1986 questionnaire and ended at the month of cholecystectomy, diagnosis of symptomatic gallstones, death, or the end of the study period, whichever occurred first. Men with asymptomatic gallstones or those whose gallstone diagnosis was not based on radiographic evidence and men with diagnosed cancer were excluded from subsequent follow-up. Thus, the eligible sample at

Table 1. **Baseline Characteristics of U.S. Men with Gallstone Disease, according to Quintile of Energy-Adjusted Polyunsaturated Fat Intake and Monounsaturated Fat Intake in 1986: The Health Professionals Follow-up Study***

Characteristic	Quintile of Polyunsaturated Fat					Quintile of Monounsaturated Fat				
	1 (lowest)	2	3	4	5 (highest)	1 (lowest)	2	3	4	5 (highest)
Participants, *n*	9185	8846	9666	8867	9192	9150	9205	9238	8961	9202
Mean age, *y*	53.8	53.7	53.7	53.7	53.8	53.9	53.7	53.7	53.7	53.8
Median intake of polyunsaturated and monounsaturated fats, *g/d*	9.0	11.3	12.8	14.6	18.4	18.7	24.2	27.3	30.3	35.6
Current smoker, %	10.9	9.9	9.1	9.4	8.4	6.2	8.1	9.6	10.4	13.3
Mean current body mass index, kg/m^2	24.8	25.0	24.9	24.9	24.9	24.3	24.7	25.0	25.2	25.4
Mean physical activity, *METS*†	20.6	19.9	19.6	19.7	19.1	25.6	21.8	19.3	17.3	15.1
Mean daily intake										
Total energy, *kcal*	1972	2003	1997	2002	1972	1950	1995	2037	2023	1941
Carbohydrate, *g*	255	240	234	228	217	275	247	233	220	197
Protein, *g*	90	93	93	94	93	91	92	92	93	94
Alcohol, *g*	15	12	11	10	9	15	13	12	10	7
Caffeine, *mg*	232	243	244	242	248	189	222	243	257	297
Cholesterol, *mg*	284	307	310	311	302	239	285	308	327	355
Saturated fat, *g*	23	24	25	25	25	17	22	25	27	31
Trans fat, *g*	2.4	2.8	2.9	3.0	3.0	1.8	2.5	2.9	3.3	3.7
Dietary fiber, *g*	21.3	20.7	20.8	20.9	21.2	26.0	21.9	20.3	19.1	17.6

* Values have been standardized for age of the cohort. METS = metabolic equivalents.
† METS/wk, defined as the energy consumed/min of sitting at rest.

risk consisted of only those who remained free of gallstone disease and cancer at the beginning of each 2-year follow-up interval. Incidence rates were calculated by dividing the number of events by person-years of follow-up in each category. Relative risks were calculated as the incidence of gallstone disease among men in different categories of dietary intake of cis unsaturated fats compared with the incidence among men in the lowest intake category, with adjustment for age in 5-year categories. Age-adjusted relative risks were calculated by using the Mantel–Haenszel summary estimator (21). Multivariate relative risks were computed by using the Cox proportional hazards regression model (22). To reduce within-person variation and best represent long-term dietary intakes, we used repeated measures of diet in the analyses (23). In particular, the incidence of gallstone disease was related to the cumulative average of cis unsaturated fat consumption from all available questionnaires up to the start of each 2-year follow-up interval. In multivariate analyses, we simultaneously included intake of total energy and potential confounding covariates, including biennially updated age (1-year categories), body mass index at the beginning of each 2-year follow-up interval (5 categories), weight change during the past 2 years (5 categories), physical activity (quintiles), dietary fiber (quintiles), diabetes (yes or no), thiazide diuretics (yes or no), nonsteroidal anti-inflammatory drugs (yes or no), pack-years of smoking (6 categories), alcohol intake (5 categories), caffeine intake (quintiles), and total energy intake (quintiles). We conducted tests of linear trend across increasing categories of dietary intake of cis unsaturated fats by assigning the median intake of unsaturated fats for categories (to minimize the influence of outliers) and treating these as a single continuous variable. All relative risks are presented with 95% CIs, and all reported *P* values are 2-sided. All analyses were performed with SAS software, version 8.2 (SAS Institute, Inc., Cary, North Carolina).

Role of the Funding Sources

The funding sources had no role in the collection, analysis, or interpretation of the data or in the decision to submit the manuscript for publication.

RESULTS

Most participants in this cohort were white (>91%). We first examined the crude distribution of relevant covariates according to average levels of both polyunsaturated and monounsaturated fats consumption among the participants (**Table 1**). At baseline in 1986, the median intakes of polyunsaturated and monounsaturated fats varied nearly 2-fold between the highest and lowest quintiles in the study sample. Men with a higher intake of polyunsaturated fat consumed less carbohydrate but had higher intakes of trans fat (see Glossary) and saturated fat. Men who reported a higher polyunsaturated fat intake tended to be more sedentary, to be less likely to be current smokers, and to drink less alcohol. Men with a higher intake of monounsaturated fat had less carbohydrate and fiber but had higher intakes of trans fat and saturated fat. Men who reported a higher monounsaturated fat intake tended to drink less alcohol, were more likely to be current smokers, and were less physically active.

During 561 938 person-years of follow-up from 1986 to 2000, we documented 2323 incident cases of gallstone disease, of which 1279 required cholecystectomy. Because intakes of polyunsaturated and monounsaturated fats were associated both directly and inversely with several potential

risk factors, we analyzed their relations with gallstone disease before and after adjustment for these variables.

The relative risk among men in the highest quintile of polyunsaturated fat consumption compared with men in the lowest quintile of polyunsaturated fat consumption was 0.90 (CI, 0.79 to 1.02; *P* for trend = 0.12) in age-adjusted analysis (**Table 2**). When extreme quintiles were compared, the association became statistically significant (relative risk, 0.84 [CI, 0.74 to 0.96]; *P* for trend = 0.016) after adjustment for multiple potential confounding variables: age; body mass index; recent weight change; cigarette smoking; history of diabetes mellitus; intakes of alcohol, caffeine, and dietary fiber; physical activity; thiazide diuretics; and nonsteroidal anti-inflammatory drugs (**Table 2**). After further adjustment for intakes of saturated fat and trans fat, the relative risk did not change appreciably. The relative risk among men in the highest quintile of monounsaturated fat consumption compared with men in the lowest quintile was 1.12 (CI, 0.98 to 1.27; *P* for trend = 0.13) in age-adjusted analysis and 0.89 (CI, 0.77 to 1.04; *P* for trend = 0.045) after adjustment for multiple potential confounding variables. The association was slightly stronger (relative risk, 0.83 [CI, 0.70 to 1.00]; *P* for trend = 0.01) after additional adjustment for intakes of saturated fat and trans fat.

We next examined the relation of total cis unsaturated fat intake (by combining the intakes of polyunsaturated and monounsaturated fats) to the risk for gallstone disease. The relative risk among men in the highest quintile of cis unsaturated fat consumption compared with men in the lowest quintile was 1.03 (CI, 0.91 to 1.18; *P* for trend = 0.61) in age-adjusted analysis. The association became significant (relative risk, 0.85 [CI, 0.74 to 0.98]; *P* for trend = 0.01) after adjustment for multiple potential confounding variables. It was slightly strengthened (relative risk, 0.82 [CI, 0.69 to 0.96]; *P* for trend = 0.006) after further adjustment for intakes of saturated fat and trans fat.

To examine whether other risk factors for gallstone disease modified the association with intake of total cis unsaturated fats, we repeated the multivariate analyses within subgroups of potential confounding variables (**Table 3**). The inverse associations between intake of total cis unsaturated fats and risk for gallstone disease persisted in almost all subgroups, although they were not always statistically significant, and we found no apparent modification of the relationship. The inverse relationship seemed to be stronger among men who had a higher body mass index or a lower physical activity level.

We further examined the associations with major specific types of polyunsaturated fat and monounsaturated fat

Table 2. **Adjusted Relative Risks for Gallstone Disease, according to Quintile of Energy-Adjusted Polyunsaturated Fat and Monounsaturated Fat Intake among U.S. Men in the Health Professionals Follow-up Study, 1986–2000**

Variable	Quintile					*P* Value for Trend
	1 (lowest)	2	3	4	5 (highest)	
Polyunsaturated fat						
Intake, *g**	9.3	11.4	12.8	14.3	17.7	
Relative risk (95% CI)						
Age-adjusted†	1.0	1.00 (0.88–1.14)	0.92 (0.81–1.05)	0.98 (0.86–1.11)	0.90 (0.79–1.02)	0.12
Multivariate‡	1.0	0.97 (0.86–1.10)	0.88 (0.78–1.01)	0.94 (0.82–1.07)	0.84 (0.74–0.96)	0.016
Additional adjustment for saturated fat and trans fat	1.0	0.96 (0.85–1.10)	0.87 (0.77–0.99)	0.93 (0.81–1.06)	0.84 (0.73–0.96)	0.01
Monounsaturated fat						
Intake, *g**	19.1	24.3	27.2	30.0	35.0	
Relative risk (95% CI)						
Age-adjusted†	1.0	1.10 (0.96–1.25)	1.11 (0.97–1.26)	1.10 (0.96–1.25)	1.12 (0.98–1.27)	0.13
Multivariate‡	1.0	1.04 (0.91–1.19)	1.01 (0.88–1.15)	0.95 (0.82–1.10)	0.89 (0.77–1.04)	0.04
Additional adjustment for saturated fat and trans fat	1.0	1.00 (0.86–1.15)	0.95 (0.81–1.11)	0.89 (0.75–1.06)	0.83 (0.70–1.00)	0.01
cis unsaturated fat§						
Intake, *g**	29.5	36.3	40.1	43.9	51.1	
Relative risk (95% CI)						
Age-adjusted†	1.0	1.04 (0.91–1.19)	1.05 (0.92–1.19)	1.08 (0.94–1.22)	1.03 (0.91–1.18)	>0.2
Multivariate‡	1.0	0.98 (0.85–1.11)	0.95 (0.83–1.09)	0.94 (0.82–1.07)	0.85 (0.74–0.98)	0.01
Additional adjustment for saturated fat and trans fat	1.0	0.94 (0.82–1.08)	0.91 (0.79–1.05)	0.89 (0.77–1.04)	0.82 (0.69–0.96)	0.006

* Values for intake were medians for each quintile, computed as the cumulative updated average.
† Relative risk adjusts for age in 5-year categories.
‡ Multivariate model included the following: age (1-year categories), time periods (1986–1988, 1988–1990, 1990–1992, 1992–1994, 1994–1998, 1998–2000), body mass index at the beginning of each 2-year follow-up interval (5 categories), weight change during the past 2 years (5 categories), physical activity (quintiles), dietary fiber (quintiles), diabetes (yes or no), thiazide diuretics (yes or no), nonsteroidal anti-inflammatory drugs (yes or no), pack-years of smoking (6 categories), alcohol intake (5 categories), caffeine intake (quintiles), and total energy intake (quintiles).
§ Sum of polyunsaturated and monounsaturated fats.

Table 3. **Multivariate Relative Risks for Gallstone Disease, Stratified by Potential Confounders according to Quintile of Intake of Total cis Unsaturated Fats***

Variable	Relative Risk (95% CI)					*P* Value for Trend
	Quintile					
	1 (lowest)	2	3	4	5 (highest)	
Alcohol intake†						
High	1.00	1.00 (0.82–1.22)	0.81 (0.65–1.01)	0.87 (0.69–1.09)	0.86 (0.67–1.09)	0.14
Low	1.00	0.91 (0.75–1.11)	1.02 (0.84–1.25)	0.96 (0.78–1.18)	0.84 (0.67–1.04)	0.05
Caffeine intake†						
High	1.00	0.96 (0.77–1.12)	0.89 (0.71–1.12)	0.89 (0.71–1.13)	0.81 (0.64–1.04)	0.05
Low	1.00	0.89 (0.74–1.07)	0.91 (0.76–1.11)	0.86 (0.71–1.06)	0.78 (0.63–0.97)	0.03
Physical activity† (METS)‡						
High	1.00	0.96 (0.78–1.18)	0.90 (0.73–1.13)	0.95 (0.75–1.20)	0.89 (0.69–1.15)	>0.2
Low	1.00	0.89 (0.74–1.08)	0.88 (0.72–1.07)	0.84 (0.69–1.03)	0.75 (0.61–0.93)	0.001
Body mass index						
≥25 kg/m^2	1.00	0.91 (0.75–1.09)	0.88 (0.73–1.07)	0.83 (0.68–1.01)	0.74 (0.60–0.92)	0.004
<25 kg/m^2	1.00	0.97 (0.78–1.21)	0.98 (0.78–1.24)	1.04 (0.82–1.33)	0.99 (0.77–1.28)	>0.2
Age†						
≥53 y	1.00	0.97 (0.82–1.14)	0.89 (0.75–1.06)	0.90 (0.76–1.08)	0.79 (0.65–0.95)	0.02
<53 y	1.00	0.87 (0.67–1.14)	0.94 (0.72–1.24)	0.87 (0.65–1.15)	0.86 (0.64–1.16)	0.10
Weight change within past 2 y						
>4 lb	1.00	0.85 (0.58–1.27)	0.70 (0.47–1.05)	0.68 (0.45–1.04)	0.77 (0.50–1.18)	0.11
≤4 lb	1.00	0.97 (0.80–1.18)	0.95 (0.77–1.16)	0.89 (0.72–1.11)	0.81 (0.64–1.03)	0.02
Current smoking						
Yes	1.00	0.87 (0.72–1.04)	0.84 (0.69–1.02)	0.82 (0.67–1.00)	0.76 (0.61–0.94)	0.008
No	1.00	1.03 (0.83–1.27)	1.02 (0.82–1.27)	0.96 (0.76–1.21)	0.87 (0.68–1.12)	0.16

* Multivariate model included the following: age (1-year categories), time periods (1986–1988, 1988–1990, 1990–1992, 1992–1994, 1994–1998, 1998–2000), body mass index at the beginning of each 2-year follow-up interval (5 categories), weight change during the past 2 years (5 categories), physical activity (quintiles), dietary fiber (quintiles), diabetes (yes or no), thiazide diuretics (yes or no), nonsteroidal anti-inflammatory drugs (yes or no), pack-years of smoking (6 categories), alcohol intake (5 categories), caffeine intake (quintiles), saturated fat intake (quintiles), trans fat intake (quintiles), and total energy intake (quintiles). The variable used for stratification was not included in the model. METS = metabolic equivalent.
† Median values were used as cutoff point.
‡ METS/wk, defined as a multiple of metabolic equivalent of sitting at rest.

(total n-3 and n-6 fatty acids) on the risk for gallstone disease (**Table 4**). Linoleic acid, the predominant dietary polyunsaturated fatty acid, was significantly associated with decreased risk for gallstone disease. The relative risk among men in the highest quintile of linoleic acid consumption compared with men in the lowest quintile was 0.87 (CI, 0.71 to 0.99; P for trend = 0.04) in multivariate analysis after adjustment for multiple potential confounding variables. It was slightly stronger (relative risk, 0.84 [CI, 0.73 to 0.96]; P for trend = 0.02) after further adjustment for intakes of saturated fat and trans fat. Intake of α-linolenic acid, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), arachidonic acid, and total n-3 and n-6 fatty acids was unrelated to the risk. Oleic acid, the predominant dietary monounsaturated fatty acid, was inversely related to the risk for gallstone disease. The relative risk among men in the highest quintile of oleic acid consumption compared with men in the lowest quintile was 0.88 (CI, 0.76 to 1.02; P for trend = 0.07) in multivariate analysis after adjustment for multiple potential confounding variables. It was strengthened (relative risk, 0.81 [CI, 0.68 to 0.98]; P for trend = 0.02) after further adjustment for intakes of saturated fat and trans fat. Palmitoleic acid was not significantly associated with the risk in the multivariate analysis.

DISCUSSION

In this large prospective study, a high intake of cis unsaturated fats was associated with a lower risk for gallstone disease in men. The inverse association was consistently present in subgroups of potential confounding variables, which suggested no apparent effect modification. We found this inverse association for both polyunsaturated and monounsaturated fats. We also found an inverse relation between intakes of linoleic acid and oleic acid, the predominant cis unsaturated fatty acids in U.S. diets, and the risk for gallstone disease.

Types of dietary fat can influence bile lithogenicity and cholesterol gallstone formation (24, 25), although the mechanism by which fats alter gallstone formation has been open to question. In animals, dietary fats rich in cis unsaturated fatty acids relative to saturated fatty acids pro-

Table 4. **Adjusted Relative Risks for Gallstone Disease, according to Quintile of Energy-Adjusted Specific Polyunsaturated Fat and Monounsaturated Fat Intake among U.S. Men in the Health Professionals Follow-up Study, 1986–2000**

Variable	Quintile					*P* Value for Trend
	1 (lowest)	2	3	4	5 (highest)	
Linoleic acid						
Intake, *g**	7.7	9.7	11.0	12.4	15.6	
Relative risk (95% CI)						
Age-adjusted†	1.00	0.97 (0.85–1.10)	0.96 (0.85–1.10)	0.94 (0.83–1.07)	0.92 (0.81–1.05)	0.20
Multivariate‡	1.00	0.94 (0.83–1.07)	0.93 (0.81–1.06)	0.90 (0.79–1.03)	0.87 (0.76–0.99)	0.04
Additional adjustment for saturated fat and trans fat	1.00	0.91 (0.80–1.04)	0.89 (0.78–1.02)	0.90 (0.79–1.03)	0.84 (0.73–0.96)	0.02
α-Linolenic acid						
Intake, *g**	0.79	0.96	1.07	1.20	1.49	
Relative risk (95% CI)						
Age-adjusted†	1.00	1.11 (0.98–1.26)	0.95 (0.83–1.09)	1.10 (0.97–1.25)	1.00 (0.87–1.14)	>0.2
Multivariate‡	1.00	1.08 (0.95–1.23)	0.91 (0.79–1.04)	1.03 (0.91–1.18)	0.92 (0.80–1.05)	>0.2
Additional adjustment for saturated fat and trans fat	1.00	1.08 (0.95–1.23)	0.90 (0.79–1.04)	1.03 (0.90–1.17)	0.92 (0.80–1.05)	>0.2
Eicosapentaenoic acid						
Intake, *g**	0.02	0.05	0.09	0.13	0.28	
Relative risk (95% CI)						
Age-adjusted†	1.00	1.07 (0.94–1.22)	0.92 (0.81–1.05)	0.89 (0.78–1.01)	0.92 (0.81–1.05)	>0.2
Multivariate‡	1.00	1.09 (0.95–1.24)	0.96 (0.84–1.09)	0.95 (0.83–1.08)	0.98 (0.86–1.12)	>0.2
Additional adjustment for saturated fat and trans fat	1.00	1.08 (0.95–1.23)	0.95 (0.84–1.09)	0.94 (0.82–1.08)	0.98 (0.86–1.13)	>0.2
Docosahexaenoic acid						
Intake, *g**	0.06	0.11	0.17	0.24	0.44	
Relative risk (95% CI)						
Age-adjusted†	1.00	1.06 (0.93–1.21)	0.98 (0.86–1.12)	0.89 (0.78–1.03)	0.97 (0.85–1.10)	0.10
Multivariate‡	1.00	1.08 (0.95–1.23)	1.02 (0.89–1.16)	0.95 (0.83–1.09)	1.02 (0.89–1.17)	>0.2
Additional adjustment for saturated fat and trans fat	1.00	1.08 (0.95–1.23)	1.01 (0.89–1.16)	0.94 (0.82–1.08)	1.02 (0.89–1.18)	>0.2
Arachidonic fatty acid						
Intake, *g**	0.10	0.14	0.16	0.19	0.25	
Relative risk (95% CI)						
Age-adjusted†	1.00	1.07 (0.94–1.22)	1.03 (0.91–1.17)	1.00 (0.88–1.15)	1.07 (0.94–1.22)	>0.2
Multivariate‖	1.00	1.04 (0.91–1.19)	1.00 (0.88–1.13)	0.95 (0.83–1.09)	0.96 (0.84–1.10)	>0.2
Additional adjustment for saturated fat and trans fat	1.00	1.04 (0.91–1.19)	0.99 (0.87–1.13)	0.95 (0.83–1.08)	0.96 (0.84–1.10)	>0.2
n-3 fatty acids						
Intake, *g**	0.93	1.18	1.37	1.58	2.08	
Relative risk (95% CI)						
Age-adjusted†	1.00	1.00 (0.88–1.14)	0.95 (0.83–1.08)	0.95 (0.83–1.08)	1.01 (0.89–1.15)	>0.2
Multivariate‡	1.00	1.01 (0.88–1.15)	0.95 (0.83–1.08)	0.94 (0.82–1.07)	0.98 (0.86–1.12)	>0.2
Additional adjustment for saturated fat and trans fat	1.00	1.01 (0.89–1.15)	0.96 (0.84–1.09)	0.95 (0.83–1.08)	1.00 (0.88–1.14)	>0.2
n-6 fatty acids						
Intake, *g**	7.7	9.8	11.3	12.9	16.3	
Relative risk (95% CI)						
Age-adjusted†	1.00	0.99 (0.87–1.12)	0.96 (0.84–1.09)	0.97 (0.85–1.11)	0.97 (0.85–1.11)	>0.2
Multivariate‡	1.00	0.96 (0.84–1.09)	0.92 (0.80–1.05)	0.91 (0.80–1.04)	0.91 (0.79–1.03)	0.19
Additional adjustment for saturated fat and trans fat	1.00	0.95 (0.83–1.08)	0.90 (0.79–1.03)	0.90 (0.79–1.03)	0.89 (0.78–1.02)	0.16
Oleic acid						
Intake, *g**	17.3	22.2	25.0	27.6	32.4	
Relative risk (95% CI)						
Age-adjusted†	1.00	1.14 (1.00–1.30)	1.06 (0.93–1.21)	1.13 (0.96–1.29)	1.10 (0.96–1.25)	0.13
Multivariate‡	1.00	1.08 (0.95–1.23)	0.96 (0.83–1.10)	0.98 (0.85–1.12)	0.88 (0.76–1.02)	0.07
Additional adjustment for saturated fat and trans fat	1.00	1.03 (0.89–1.19)	0.90 (0.77–1.05)	0.91 (0.77–1.07)	0.81 (0.68–0.98)	0.02
Palmitoleic acid						
Intake, *g**	0.9	1.2	1.4	1.6	2.1	
Relative risk (95% CI)						
Age-adjusted†	1.00	1.05 (0.93–1.20)	1.04 (0.91–1.19)	1.15 (1.01–1.31)	1.19 (1.05–1.36)	0.004
Multivariate‡	1.00	1.00 (0.87–1.14)	0.94 (0.82–1.08)	1.00 (0.87–1.15)	0.94 (0.82–1.09)	>0.2
Additional adjustment for saturated fat and trans fat	1.00	0.98 (0.85–1.13)	0.92 (0.79–1.07)	0.98 (0.84–1.15)	0.92 (0.78–1.10)	>0.2

* Values for intake were medians for each quintile, computed as the cumulative updated average.

† Relative risk adjusts for age in 5-year categories.

‡ Multivariate model included the following: age (1-year categories), time periods (1986–1988, 1988–1990, 1990–1992, 1992–1994, 1994–1998, 1998–2000), body mass index at the beginning of each 2-year follow-up interval (5 categories), weight change during the past 2 years (5 categories), physical activity (quintiles), dietary fiber (quintiles), diabetes (yes or no), thiazide diuretics (yes or no), nonsteroidal anti-inflammatory drugs (yes or no), pack-years of smoking (6 categories), alcohol intake (5 categories), caffeine intake (quintiles), and total energy intake (quintiles).

tect against gallstone formation (10). Supplementation of the diet with oleic or linoleic acid resulted in the incorporation of these fatty acids into biliary phosphatidylcholines in hamster bile (10), of which the major phosphatidylcholine species are identical to those found in human bile (26), and resulted in more stabilized cholesterol–phospholipid vesicles. Phosphatidylcholines make up more than 90% of biliary phospholipids (27). Thus, by modifying the molecular species of phosphatidylcholine, the addition of unsaturated fatty acids to the diet in hamsters decreased bile lithogenicity and prevented cholesterol gallstone formation (10). Another experiment in hamsters demonstrated that substitution of olive oil or corn oil for butter fat prevented gallstone formation completely, whereas palmitic acid added to the diet enhanced cholesterol gallstone formation (9). A study in African green monkeys showed that feeding of a fish oil–supplemented diet high in polyunsaturated fatty acids led to decreased cholesterol saturation in bile and was associated with a lower rate of gallstone formation (28).

In addition, in metabolic studies, increased intake of polyunsaturated and monounsaturated fats can improve insulin sensitivity (7, 29) and thereby may decrease the incidence of gallstone formation (6). One proposed mechanism for the effect on insulin sensitivity comes from observations that the fatty acid composition of cell membranes, which reflects the fatty acid composition of the diet, modulates insulin action (30); a greater saturated fatty acid content of membrane phospholipids increases insulin resistance, whereas a greater polyunsaturated or monounsaturated fatty acid content can improve insulin sensitivity. Insulin resistance itself seems to be a risk factor for gallstones (6). Gallstone formation could be precipitated by hyperinsulinemia, which may increase the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase, the rate-limiting enzyme in hepatic synthesis of new cholesterol (31, 32). Our results may support this hypothesis in that the inverse relationship between unsaturated fat intake and risk for gallstone disease seemed to be stronger among men who had a higher body mass index or a lower physical activity level that is predisposed to insulin resistance.

Epidemiologic or clinical studies on dietary intake of polyunsaturated or monounsaturated fat and risk for gallstone disease are sparse and divergent. In a case–control study in Greece, high consumption of olive oil, a major source of oleic acid, was inversely associated with gallstone disease (33). In a case–control study in Spain, patients with gallstones were shown to have significantly higher intake of monounsaturated fatty acids than controls (12), but in a population-based case–control study in southern Italy, dietary monounsaturated fat was inversely associated with risk for gallstone formation (34). In a cross-sectional study among Jews in Tel Aviv and Arabs in Gaza, the consumption of unsaturated fats was greater among Arabs in Gaza who had a lower prevalence of gallstones (35). These results may be limited by the lack of long-term dietary information, nonvalidated assessment of nutrients, suboptimal study design, or small sample size.

The prospective design of our study avoids the potential bias for differential recall of intake by gallstone cases and noncases because all data on food were collected before the diagnosis of gallstone disease. In addition, consistently high follow-up rates reduce the possibility that our results are biased by men lost to follow-up in this cohort. Thus, these potential biases should have been minimal.

The possibility of misclassification might be of concern because information on nutrient intake was collected by self-report. Random within-person variation could attenuate any true association of interest, but the semi-quantitative food-frequency questionnaire was designed to minimize this error by assessing average long-term dietary intake during the successive follow-up periods. These repeated measurements took into account possible changes in diet with time and reduced random variation in reporting. Because the data on intake of cis unsaturated fats were collected before the onset of symptoms of gallstone disease, any measurement errors would be expected to be unrelated to the end points. Thus, any nondifferential misclassification would most likely bias the relative risks toward the null hypothesis and weaken, rather than strengthen, any true relationship (21). Participants also might change their diets after developing gallstone disease, which might dilute a possible association between intake of cis unsaturated fats and risk for gallstone disease. To reduce this potential bias, we excluded participants with gallstones at baseline and stopped updating individual dietary information once a participant reported gallstone disease during the follow-up periods.

Our results were restricted to men with cholecystectomy or diagnostically confirmed but unremoved symptomatic gallstones. Silent gallstones were not included because most would have been detected incidentally. Thus, the results may not be generalizable to the entire population with gallstones. However, the study focused on clinically relevant gallstone disease.

In the study sample, no systemic diagnostic screening procedures for the presence of gallstones were performed. Considerable underascertainment of gallstones is likely because most gallstones are silent (36). It was not likely that the presence of silent gallstones at baseline was associated with the reporting. Because relative risk estimation in follow-up cohort studies would not be biased by uniform underascertainment (21), our results were not likely to be biased as a result of silent gallstones.

Our findings were consistent with the results from experimental studies that cis unsaturated fatty acids can inhibit gallstone formation. Our data demonstrated that increased total dietary cis unsaturated fat intake was associated with a significantly decreased risk for gallstone disease. The approximate 2-fold difference in the consumption of cis unsaturated fats, when highest and lowest quintiles were compared in this cohort, was associated with

an approximate 20% risk reduction in gallstone disease during 14 years of follow-up. However, as in any observational study, we cannot conclusively exclude the possibility that some unknown factor associated with high cis unsaturated fat intake might be responsible for the risk reduction. Residual confounding in this study might come from other dietary factors, but we had a comprehensive dietary assessment that could examine these. Because the sample we studied is relatively homogeneous with respect to education and occupation, confounding by socioeconomic status was minimized. Residual confounding probably could not entirely explain the observed inverse relationship (37).

In conclusion, our results suggest that a high intake of polyunsaturated and monounsaturated fats in the context of an energy-balanced diet is associated with a reduced risk for gallstone disease in men. Although the optimal amount of unsaturated fat intake is still unknown (38, 39), our findings support the notion that, in dietary practice, a higher intake of cis unsaturated fats can confer health benefits (40, 41). Our results should also have implications for additional epidemiologic, clinical, and mechanistic research.

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